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High temperature increases 2,4-D metabolism in resistant *Amaranthus palmeri*

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Research Article

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Abstract

Palmer amaranth (*Amaranthus palmeri* S. Watson) is a troublesome weed in several cropping systems in the United States. The evolution of resistance to multiple herbicides is a challenge for the management of this weed. Recently, we reported metabolic resistance to 2,4-D possibly mediated by cytochrome P450 (P450) activity in a six-way-resistant *A. palmeri* population (KCTR). Plant growth temperature can influence the herbicide efficacy and level of resistance. The effect of temperature on 2,4-D resistance in *A. palmeri* is unknown. In the present research, we investigated the response of KCTR and a known susceptible (MSS) *A. palmeri* response to 2,4-D grown under low-temperature (LT, 24/14 C, day/night [d/n]) or high-temperature (HT, 34/24 C, d/n) regimes. When MSS and KCTR plants were 8- to 10-cm tall, they were treated with 0, 140, 280, 560 (field recommended dose), 1,120, and 2,240 g ai ha^{-1} of 2,4-D. Further, 8- to 10-cm-tall MSS and KCTR plants grown at LT and HT were also treated with [^{14}C]2,4-D to assess the metabolism of 2,4-D at LT and HT. The results of dose–response experiments suggest that KCTR *A. palmeri* exhibits 23 times more resistance to 2,4-D at HT than MSS. Nonetheless, at LT, the resistance to 2,4-D in KCTR was only 2-fold higher than in MSS. Importantly, there was enhanced metabolism of 2,4-D in both KCTR and MSS *A. palmeri* at HT compared with LT. Further, treatment with the P450 inhibitor malathion, followed by 2,4-D increased the susceptibility of KCTR at HT. Overall, rapid metabolism of 2,4-D increased KCTR resistance to 2,4-D at HT compared with LT. Therefore, the application of 2,4-D when temperatures are cooler can improve control of 2,4-D–resistant *A. palmeri*.

Introduction

Management of multiple-resistant Palmer amaranth (*Amaranthus palmeri* S. Watson) is a major challenge in corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] producing areas of the U.S. Midwest, including Kansas. *Amaranthus palmeri* is highly competitive, and depending on its time of emergence and density, crop yield losses can range from 11% to 91% (Bensch et al. 2003; Massinga et al. 2019; Ward et al. 2013). There has been a steady increase in incidence of multiple herbicide resistance in *A. palmeri* (Heap 2023). Evolved resistance mechanisms to herbicides can be grouped into a) alteration to herbicide target (TSR) and/or non–target site (NTSR)-based. TSR is associated with mutations in the molecular target of the herbicide resulting in reduced affinity to herbicides or increased production of the target enzyme (Gaines et al. 2020; Murphy and Tranel 2019). NTSR mechanisms involve physiological processes such as reduced absorption, translocation, and/or increased metabolism of herbicides (Gaines et al. 2020; Jugulam and Shyam 2019; Yuan et al. 2007).

The synthetic auxinic herbicide 2,4-D is used widely to control broadleaf weeds in cereal crops (Mithila et al. 2011; Peterson et al. 2016; Song 2014). Natural tolerance to 2,4-D in monocots such as corn and wheat (*Triticum aestivum* L.) provides the opportunity to selectively control dicot weeds in these crops (Fang and Butts 1954). Evolution of resistance to these herbicides because of their extensive use has been reported in several dicot weeds, including *A. palmeri* (Heap 2023). In Kansas, resistance to 2,4-D was reported in two populations (Kumar et al. 2019; Shyam et al. 2022). Rapid metabolism of 2,4-D was attributed to the resistance in dicot weeds such as tall fleabane [*Conyza sumatrensis* (Retz.) E. Walker] (Palma-Bautista et al. 2020), waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] (Figueiredo et al. 2018), and *A. palmeri* (Shyam et al. 2022). Plants can metabolize auxins/auxinic herbicides through three pathways: direct conjugation, side-chain cleavage, and ring hydroxylation via the activity of cytochrome P450 enzymes (P450) (Eyer et al. 2016; Montgomery et al. 1971; Peterson et al. 2016). P450-mediated metabolism of 2,4-D was reported in tolerant crops as well as resistant weed species (Shergill et al. 2018; Shyam et al. 2022).

Environmental conditions such as temperature, relative humidity, soil moisture, photoperiod, and time of herbicide application have an effect on germination, growth, and post-emergence herbicide efficacy in weeds (Küpper et al. 2018; Matzrafi et al. 2016; Olson et al. 2000; Radosevich and Bayer 1979). Germination and growth of *A. palmeri* were found to be positively correlated with increase in temperature (Guo and Al-Khatib 2003). Temperature can influence herbicide efficacy by altering the herbicide uptake, translocation, or metabolism and can select for pleiotropic effects as well (Dyer 2018). Studies indicate that the efficacy of 2,4-D and dicamba was reduced at high temperatures (Johnston et al. 2018; Ou et al. 2018). High temperature decreases the efficacy of mesotrione in *A. palmeri*, primarily because of the increased metabolism of this herbicide (Godar et al. 2015). Similarly, decreased metabolism of tembotrione was found at lower temperatures in an *A. palmeri* population from Nebraska (Küpper et al. 2018). Our previous research found an increase in metabolism of 2,4-D at high temperature in an *A. tuberculatus* population (Shyam et al. 2019).

An *A. palmeri* population (KCTR) was found to exhibit metabolic resistance to five modes of action of herbicides, including 2,4-D (Shyam et al. 2021). The 2,4-D resistance in KCTR *A. palmeri* was attributed to rapid metabolism via P450 activity (Shyam et al. 2022). However, the effect of temperature on the efficacy of 2,4-D is unknown in this *A. palmeri* population. The objectives of this study were to (1) determine the effect of temperature on the level of 2,4-D resistance in KCTR *A. palmeri* compared with a known 2,4-D–susceptible biotype (MSS), (2) study the metabolic profile of [^{14}C]2,4-D in KCTR and MSS plants grown under different temperature regimes, and (3) investigate the P450 enzyme activity in metabolizing 2,4-D at low- or high-temperature conditions.

Materials and Methods

Plant Material and Growing Conditions

The same 2,4-D–resistant (KCTR) and 2,4-D–susceptible (MSS) *A. palmeri* populations that were used in our previous research (Shyam et al. 2022) were used in the present study. *Amaranthus palmeri* seeds were germinated in plastic trays (25 by 15 by 2.5 cm) filled with commercial potting mix (Pro-Mix® potting mix, Premier Horticulture, Quakertown, PA, USA). After emergence, individual seedlings were transplanted into plastic pots (9 by 6 by 5 cm), and maintained in the greenhouse at 30/20 C day/night (d/n) with 15 h of photoperiod supplemented with 120 µmol $m^{-2}s^{-1}$ illumination provided with sodium-vapor lamps at 60 ± 10% relative humidity. When plants reached the 4-leaf stage, they were transferred to two separate growth chambers set for low-temperature (LT) (24/14 C) or high-temperature (HT) (34/24 C) regimes. These temperature regimes were selected based on the average diurnal temperatures from mid-May to mid-June in Kansas, according to Kansas State University Mesonet (https://mesonet.k-state.edu/). Incandescent and fluorescent bulbs were used in growth chambers to maintain a light level of 750 µmol m^{-2} s^{-1} (15/9 h, d/n condition), and relative humidity was maintained at 60 ± 10% throughout the study. Plants were watered daily.

2,4-D Dose–Response Experiment

When the plants at LT or HT reached 8- to 10-cm in height, the dose–response experiment was performed with 2,4-D herbicide (2,4-D Amine 4, WinField Solutions, St Paul, MN, USA). Both KCTR and MSS plants were treated with 0, 140, 280, 560 (field recommended dose), 1,120, and 2,240 g ae ha^{-1} 2,4-D, without the addition of adjuvants. The herbicides were applied using a bench-type sprayer (Research Track Sprayer, Generation III, De Vries Manufacturing, Hollandale, MN, USA) equipped with a single flat-fan nozzle (80015LP TeeJet® tip, Spraying Systems, Wheaton, IL, USA) delivering 187 L ha^{-1} at 240 kPa in a single pass at 4.75 km h^{-1}. The treated plants were transferred back to their respective growth chambers 30 min after 2,4-D application. At least four to six replications were included in each treatment. At 4 wk after treatment (WAT), the visual injury relative to nontreated plants was recorded, and aboveground plant tissue was harvested, placed in paper bags, and dried in an oven at 60 C (Thelco Laboratory Oven, Precision Scientific, IL, USA) for 3 d, after which dry shoot biomass was measured. Percent dry shoot biomass was calculated relative to the nontreated control for each population for graphical visualization. The experiment was repeated.

[^{14}C]2,4-D Metabolism Experiment

The KCTR and MSS plants were grown in the greenhouse and transplanted into individual pots as described earlier and were then acclimatized in LT or HT conditions in growth chambers (as described earlier) for the 2,4-D metabolism experiment. [^{14}C]2,4-D (American Radio Chemicals, St Louis, MO, USA) dissolved in ethanol was used as a stock solution for preparing the working stock solution. The stock solution (10 µl), which contained a mixture of [^{14}C]2,4-D (0.34 kBq with a specific activity of 5.5 MBq $mmol^{-1}$) and commercial unlabeled 2,4-D amine, was applied as droplets on the fourth-youngest fully expanded leaves of 8- to 10-cm-tall KCTR and MSS *A. palmeri* plants. The amount of 2,4-D applied was equivalent to the field recommended dose (560 g ae ha^{-1}) of 2,4-D. The [^{14}C]2,4-D–treated plants were returned to their respective growth chambers 30 min after herbicide application. At 24 h after treatment (HAT), plant tissue was collected and processed for determining the metabolism of 2,4-D, following Shyam et al. (2022). The treated leaf was washed twice with 5 ml of wash solution containing 10% (v/v) ethanol and 0.5% Tween-20 in 20-ml scintillation vials for 1 min to remove unabsorbed 2,4-D from the leaf surface. Then 15 ml of the scintillation cocktail (Ecolite-(R), MP Biomedicals, Santa Ana, CA, USA) was added to the leaf rinsate to measure the radioactivity using a liquid scintillation counter (Beckman Coulter LS6500 Liquid Scintillation Counter, Beckman Coulter, Fullerton, CT, USA) to ensure the absorption of herbicide.

Aboveground plant tissue, including the treated leaf, was wrapped in aluminum foil and flash-frozen in liquid nitrogen and stored at −80 C until processing. The frozen plant tissue was ground to a fine powder using liquid nitrogen in a mortar and pestle and transferred to a centrifuge tube. Fifteen mL of 90% aqueous acetone was added to the centrifuge tube. Samples were incubated at 4 C for at least 16 h for extraction. The extract was centrifuged at 5000 × *g* for 10 min, and the supernatant was transferred to a new centrifuge tube and concentrated at 45 C for 1.5 to 2 h with a rotary evaporator (Centrivap, Labconco, Kansas City, MO, USA). The final volume of the supernatant was maintained at around 600 µl and transferred to a 1.5-ml microcentrifuge tube and centrifuged at 15,000 × *g* for 10 min. The supernatant containing the radioactivity was measured with the liquid scintillation counter and normalized to 60 dpm ml^{-1} by diluting the samples with 50% acetonitrile (1:1 v/v acetonitrile: water). The final solution was analyzed using reverse-phase high-performance liquid chromatography (HPLC; Agilent Technologies, Santa Clara, CA, USA) with a Zorbax SB-C18 column (4.6 by

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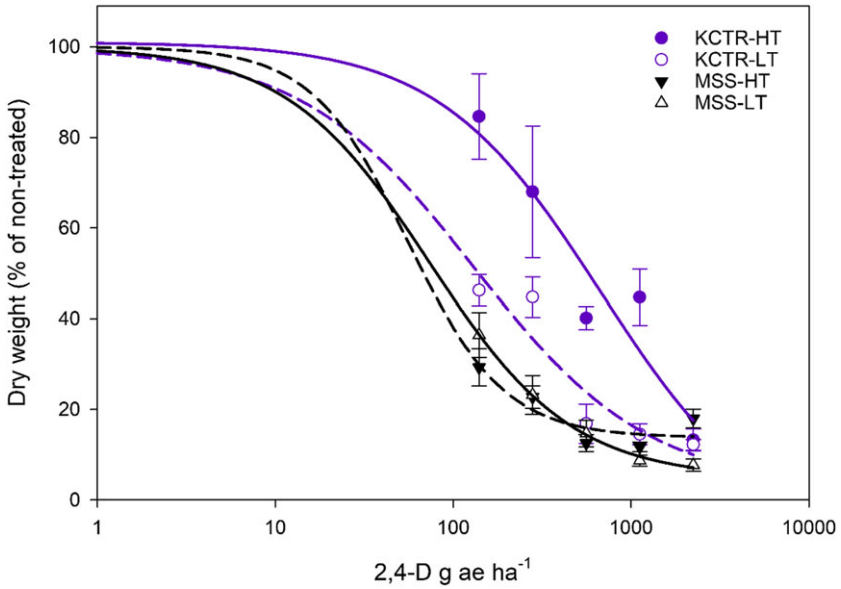

Figure 1. The 2,4-D dose–response of *Amaranthus palmeri* populations susceptible (MSS) and resistant (KCTR) to 2,4-D under low (LT; 24/14 C) and high (HT; 34/24 C) temperature regimes at 4 wk after treatment. Regression analysis of dry shoot biomass was fit using the four-parameter log-logistic model (Equation 1). The experiment was carried out in two runs with a total of four to five biological replicates.

250 mm, 5-µm particle size; Agilent Technologies) to resolve the contents into parent [^{14}C]2,4-D and its metabolites. From the normalized solution, 50 µl was set as the injection volume. Eluent A consisted of 0.1% (v/v) trifluoroacetic acid (TFA) in water, and eluent B had 0.1% TFA in acetonitrile. The following elution profile was used: 0% B at 0 min; 20% B (v/v) at 1 min; 40% B at 3 min; 60% B at 7 min; 70% B at 19 min; 90% B at 21 min; 40% B at 23 min and 0% B at 25 min (25 min total). The radioactivity was measured by radioactive detector (EG&G Berthold, LB 509) employed with an admixture flow cell, Z-1000 (Berthold Technologies GmbH, Bad Wildbad, Germany) at a flow rate of 1 ml min^{-1} of scintillation fluid, Ultima-Flo AP cocktail (Perkin-Elmer, Waltham, MA, USA). The percent parent [^{14}C]2,4-D remaining in each sample was determined based on the peak areas of the percent of [^{14}C]2,4-D relative to the total extractable radioactivity. The experiment included at least four replicates and was repeated once.

P450 Inhibitor Experiment

A known P450 inhibitor, malathion, was used to determine the function of P450 enzymes in metabolizing 2,4-D. Both MSS and KCTR *A. palmeri* plants were grown (as described earlier) in two separate growth chambers maintained for LT or HT regimes. When the plants reached 8 to 10 cm, they were treated with malathion at 1,500 g ai ha^{-1} with 0.25% nonionic surfactant (Activate Plus™, WinField Solutions) 30 min before foliar application of 2,4-D. Additionally, 50 ml pot^{-1} of 5 mM malathion was applied at 48 HAT. These concentrations and time of application of malathion were selected based on our previous work (Shyam et al. 2022). Aboveground plant tissue was harvested at 4 WAT and dried at 60 C for a week, and dry biomass was measured. At least four replications were included in each treatment, and the experiment was repeated once.

Statistical Analysis

All the experiments were conducted in a completely randomized design and repeated. Dose–response data (expressed as a percentage of the untreated control) were analyzed using the DRC package in R v. 4.1.2 (Knezevic et al. 2007; Ritz et al. 2015). A four-parameter log-logistic model was used to show the relationship between herbicide rate and response:

$$y = c + \{d - c/1 + \exp[b(\log x - \log e)]\} \quad [1]$$

where d is the asymptotic value of y at the upper limit, c is the lower asymptotic limit, b is the slope of the curve around e, where e is GR_{50} (the herbicide rate giving response halfway between d and the lower asymptotic limit c), and x is the herbicide dose. The suitability of the four-parameter log-logistic model was verified using the *modelFit* function in the DRC package. The resistance index (R/S) was calculated as the GR_{50} ratio for the KCTR and MSS populations.

The metabolism data were analyzed using one-way ANOVA, and the means were compared using Tukey's honestly significant different (HSD) test ($\alpha = 0.05$). The effect of malathion on 2,4-D metabolism was analyzed using a factorial ANOVA in R, and contrast comparisons were adjusted using Tukey's HSD test. The data were plotted using the GGPLOT2 package for graphical visualizations. Homogeneity of variance via Levene's test ($\alpha = 0.05$) was carried out in R using the CAR package. No significant difference between runs was found, so the data were combined and analyzed together.

Results and Discussion

2,4-D Dose Response

The 2,4-D dose–response results suggest varying response of KCTR and MSS *A. palmeri* under HT and LT regimes. At 4 WAT, the 2,4-D doses required to reduce 50% of growth (GR_{50}) of KCTR and MSS plants at HT were 1,307 and 56.5 g ha^{-1}, respectively, whereas at LT they were 130 and 62.6 g ha^{-1}, respectively (Figure 1; Table 1), suggesting that the resistance indices for KCTR relative to MSS at HT and LT were 23 and 2, respectively (Table 1).

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Table 1. Estimates of regression parameters from the whole-plant 2,4-D dose–response study of *Amaranthus palmeri* populations susceptible (MSS) and resistant (KCTR) to 2,4-D grown under low- and high-temperature regimes based on dry shoot biomass collected at 4 wk after treatment.[a]

Population	Day/night temperature C	GR_{50} (SE)	R/S	Regression parameters	
		g ha^{-1}		*b* (SE)	*d* (SE)
KCTR	24/14	130.7 (36.3)	2	0.89 (0.36)	99.8 (4.9)
	34/24	1307.5 (3850)	23	0.67 (0.4)	106.6 (6.9)
MSS	24/14	62.55 (21.9)	1	1.37 (0.58)	100 (3.2)
	34/24	56.8 (25)	1	1.64 (0.7)	99.9 (4.07)

[a]Data were combined over two experimental runs. Values in parentheses are standard errors of the mean. Resistance index (R/S) is the ratio of the GR_{50} of the KCTR population to that of MSS population.

No significant difference in GR_{50} of MSS was observed when grown under HT or LT regimes. However, MSS *A. palmeri* was controlled with lower doses of 2,4-D at LT than HT (Supplementary Figure S1A and C). In contrast, KCTR plants grown at HT survived even the highest dose of 2,4-D, although they showed more injury when grown at LT (Supplementary Figure S1B and D). The data indicated that the KCTR population exhibits a high level of resistance to 2,4-D at HT compared with LT. A previous study demonstrated that the level of resistance to 2,4-D in the KCTR population was 6 to 11 times more than that of the MSS population when grown under a temperature regime of 32.5/22.5 C (Shyam et al. 2022). In the present study, when 2,4-D-dose response experiments were conducted at HT using the same KCTR and MSS plants, the KCTR showed 23-fold resistance relative to MSS, whereas at LT, the resistance index was only 2-fold (Figure 1; Table 1). A similar increase in resistance level was observed between 2,4-D–resistant and 2,4-D–susceptible *A. tuberculatus*, where a 20-fold resistance level was recorded at high temperature (34/20 C) compared with 9-fold at low temperature (24/10 C) (Shyam et al. 2019). *Amaranthus palmeri* also showed decreased sensitivity to mesotrione at HT compared with LT (Godar et al. 2015). Similarly, *A. tuberculatus* and large crabgrass [*Digitaria sanguinalis* (L.) Scop.] showed 6- to 7-fold resistance to mesotrione at 32 C compared with 18 C (Johnson and Young 2002). Conversely, common cocklebur (*Xanthium strumarium* L.) and velvetleaf (*Abutilon theophrasti* Medik.) showed 3-fold susceptibility to mesotrione at 32 C compared with 18 C (Johnson and Young 2002). On the other hand, Ganie et al. (2017) found an improved efficacy of 2,4-D and glyphosate in controlling common ragweed (*Ambrosia artemisiifolia* L.) and giant ragweed (*Ambrosia trifida* L.) at high temperatures. Thus, high temperatures can reduce herbicide efficacy; however, this effect is also dependent on the weed species and mode of action of the herbicide. Our data suggest HT significantly reduced the efficacy of 2,4-D in KCTR *A. palmeri*. As the other conditions, such as moisture, relative humidity, and light intensity were kept constant at HT and LT in this study, it is likely that temperature influenced the efficacy of 2,4-D in controlling *A. palmeri*.

[^{14}C]2,4-D Metabolism

To study the effect of temperature on 2,4-D metabolism, we carried out an HPLC-based analysis of *A. palmeri* grown under HT and LT regimes. [^{14}C]2,4-D as a parent compound was detected at a retention time of 10.9 min, and the polar metabolites of 2,4-D were identified at 5.9, 6.5, 7.5, and 8.2 min (Figure 2). The HPLC chromatograms illustrate higher peaks of the parent [^{14}C]2,4-D in plants grown at LT (Figure 2A and B) compared with HT (Figure 2C and D), indicating more metabolism of 2,4-D at HT than LT. About 94.5% of the total absorbed 2,4-D was present as herbicidally active parent [^{14}C]2,4-D in MSS plants grown at LT, whereas it was 83% at HT (Figure 3). KCTR plants grown at LT retained 68.2% of the parent [^{14}C]2,4-D compared with 39% at HT ($P < 0.01$) (Figure 3). The overall comparison of the effect of temperature on the amount of parent [^{14}C]2,4-D retained showed a significant difference between KCTR and MSS plants ($P = 0.01$). Our data suggest enhanced metabolism of 2,4-D in plants grown at HT compared with LT in both MSS and KCTR *A. palmeri*.

P450 enzymes play a major role in xenobiotic detoxification and are involved in the chemical transformations of various herbicides (Aarthy et al. 2022; Dimaano and Iwakami 2021). The auxinic herbicide-tolerant plants detoxify 2,4-D through different mechanisms, such as amino acid conjugation (Eyer et al. 2016), side-chain cleavage, and ring hydroxylation (Peterson et al. 2016). The principal route of 2,4-D detoxification in wheat includes aryl hydroxylation and subsequent glucose conjugation to oxidized products (Frear 1995). Similarly, hydroxylated metabolites of 2,4-D were reported in resistant corn poppy (*Papaver rhoeas* L.) populations (Torra et al. 2017). A multiple-resistant *A. tuberculatus* population from Missouri exhibited 7- to 9-fold faster 2,4-D metabolism, possibly mediated by P450 enzymes, compared with the susceptible population (Shergill et al. 2018). The KCTR *A. palmeri* plants metabolize [^{14}C]2,4-D into polar compounds within 48 HAT (Shyam et al. 2022).

In this study, the KCTR plants metabolized ~61% of the absorbed [^{14}C]2,4-D at HT compared with only 31% at LT (Figures 2 and 3). Similarly, susceptible MSS plants showed 17% metabolism of [^{14}C]2,4-D at HT compared with only 5% at LT (Figure 3). Abiotic factors such as temperature can affect systemic acquired resistance, which has been proposed to regulate proteins involved in functions such as repair, defense, and NTSR mechanisms, but the exact mechanism is unknown (Dyer 2018). A similar effect of decreased sensitivity to mesotrione was found in an *A. palmeri* population at high temperature (Godar et al. 2015). In corn and broadleaf signalgrass [*Urochloa platyphylla* (Munro ex C. Wright) R.D. Webster] absorption, translocation, and metabolism of primisulfuron and nicosulfuron were rapid at warm temperatures (30/20 C) compared with low temperatures (20/10 C) (Gallaher et al. 1999). Therefore, NTSR-based mechanisms appear to alter the level of resistance at higher temperatures. Importantly, the metabolic resistance to herbicides in weed species is likely to be affected by temperature alterations.

P450 Inhibitor Assay

To evaluate the role of P450 enzymes in metabolism of 2,4-D at HT, we performed a P450-inhibitor experiment using malathion. Treatment with malathion alone did not impact any reduction in biomass of KCTR *A. palmeri* grown at LT or HT. However, there

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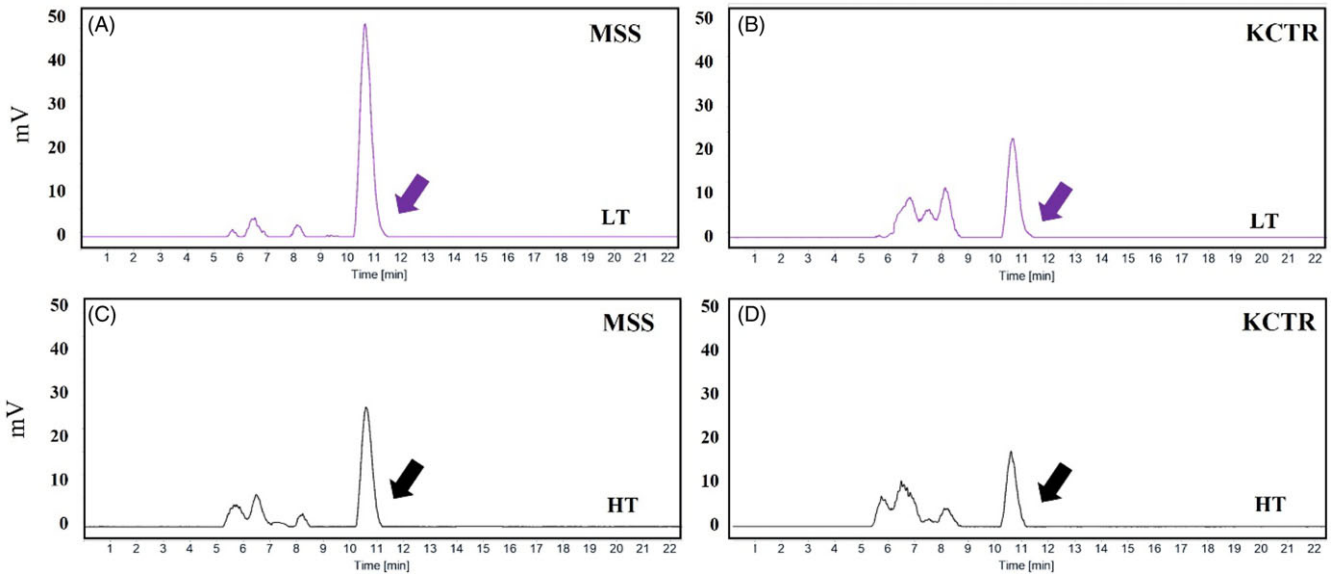

Figure 2. Chromatograms illustrating metabolism of 2,4-D. The parent compound [^{14}C]2,4-D eluted at retention time of 10.5 (marked with an arrow) along the three 2,4-D metabolite peaks identified at 6, 7, and 8 min in *Amaranthus palmeri* populations (A and C) susceptible (MSS) and (B and D) resistant (KCTR) to 2,4-D at 24 h after treatment (HAT) under (A and B) low-temperature (LT; 24/14 C, d/n) and (C and D) high-temperature (HT; 34/24 C, d/n) regimes. A higher peak of herbicidally active parent 2,4-D was observed at LT (A and B) compared with HT, indicating more metabolism at HT. Data were combined over two experimental runs with six to eight replicates in total.

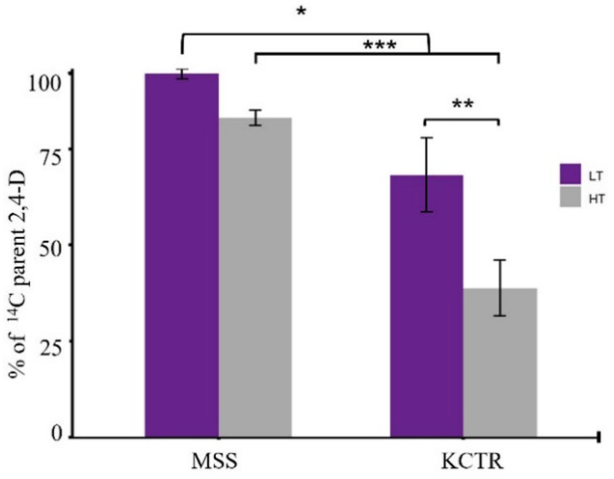

Figure 3. Percentage of [^{14}C]2,4-D parent compound in *Amaranthus palmeri* populations (A) susceptible (MSS) and (B) resistant (KCTR) to 2,4-D at 24 h after treatment (HAT) under low-temperature (LT; 24/14 C, d/n) and high-temperature (HT; 34/24 C, d/n) regimes. Data were combined over two experimental runs. *P-value = 0.01, **P-value < 0.01, ***P-value < 0.0001, indicates the level of significance of difference in means, and error bars represent standard errors of the mean for six to eight biological replicates.

was a biomass reduction of 20% to 25% in MSS plants treated with malathion alone compared with the nontreated control at LT or HT (Figure 4A and B). But treatment with 2,4-D plus malathion reduced the biomass accumulation of KCTR compared with 2,4-D alone both at LT and HT, although the difference is not statistically significant (Figure 4). Similar results were also found in MSS *A. palmeri* as well (Figure 4). Although we selected the malathion dosage based on previous work (Shyam et al. 2022), it is possible that this dose needs to be adjusted, considering that the experiments presented here were conducted at different temperatures than those used in Shyam et al. (2022). A decrease in sensitivity to the acetyl-CoA carboxylase inhibitors diclofop-methyl and pinoxaden was reported at high temperatures (34/28 C) due to P450-based metabolism in a ryegrass (*Lolium* spp.) population (Matzrafi et al. 2016). Slower metolachlor metabolism was found in corn grown at LT (21/13 C) when compared to HT (30/21 C) (Viger et al. 1991). The interactions between the enzyme and ligand (ultimately the efficiency of enzymatic reaction) are determined by thermal dependencies that vary across plant species (Mahan et al. 1990). In addition to metabolism, temperature was also found to have an effect on the translocation ability of the synthetic auxins (Radosevich and Bayer 1979). Decreased translocation of dicamba was attributed to the reduced efficacy of dicamba in kochia [*Bassia scoparia* (L.) A.J. Scott] at HT (Ou et al. 2018).

Climate change leading to altered environmental conditions increases the risk for metabolism-based reduction in herbicide efficacy for weed control (Matzrafi et al. 2016). The efficacy of auxinic herbicides for controlling dicotyledonous weeds depends on several environmental factors, including temperature and time of application of herbicides (Johnston et al. 2018; Stewart et al. 2009). The current study was carried out to determine the efficacy of 2,4-D in controlling *A. palmeri* under different temperature regimes and to understand the effect of temperature on 2,4-D metabolism. A distinct variation in the efficacy of 2,4-D was found in *A. palmeri* grown at HT versus LT. At HT, 2,4-D control of the resistant *A. palmeri* population decreased because of the enhanced 2,4-D metabolism. The results suggest that the application of 2,4-D at low air-temperature conditions can help manage *A. palmeri* regardless of its resistance or susceptibility to this herbicide. With frequent episodes of temperature fluctuation during the cropping season, weed management can be more challenging, and prudent strategies need to be followed for the management of weeds under varied climatic conditions.

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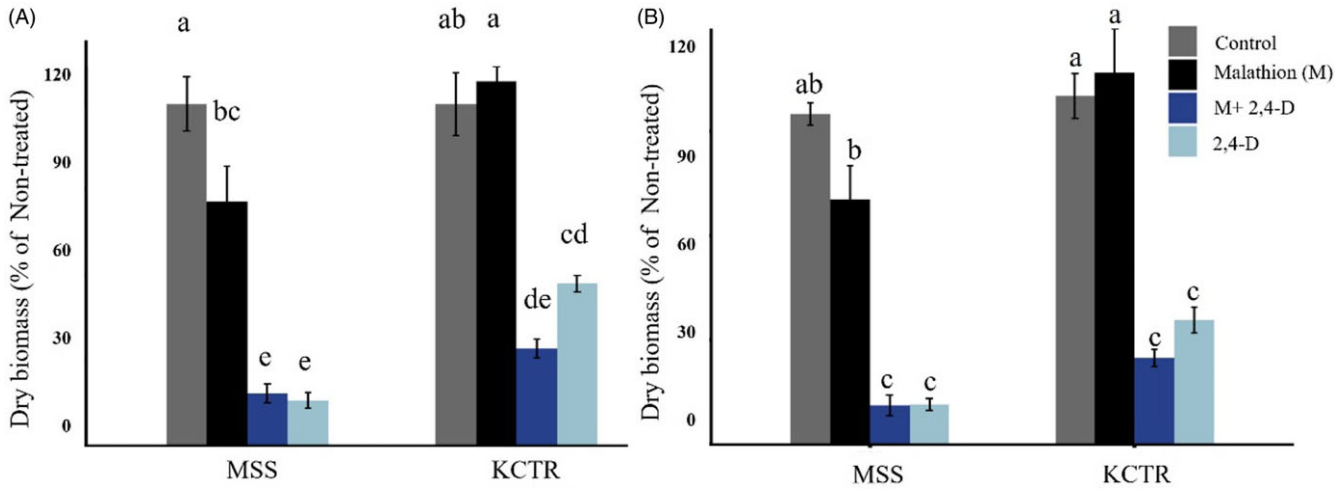

Figure 4. Effect of cytochrome P450 inhibitor malathion on 2,4-D herbicide metabolism in *Amaranthus palmeri* populations susceptible (MSS) and resistant (KCTR) to 2,4-D under (A) high-temperature (HT; 34/24 C, d/n) and (B) low-temperature (LT; 24/14 C, d/n) regimes. Error bars represent standard errors of the mean of four to six biological replicates, and the experiment was performed twice. Letters represent significant differences identified by the separation of means using Tukey's honestly significant different test.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/wsc.2023.26

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